

WHAT IS CLAIMED IS:

1. A method to identify a ligand site obeying a two-state or more complex binding behavior in a transient complex of a ligand with a target molecule, said method comprising the steps of:
 - a) preparing a ligand with at least one nucleus detectable by NMR;
 - b) collecting NMR relaxation dispersion profiles for said nucleus at two or more magnetic fields;
 - c) determining apparent transverse relaxation rates from said dispersion profiles of step b);
 - d) assigning resonance peaks to said nucleus of the ligand with one- and/or multi-dimensional NMR;
 - e) contacting the ligand of step a) with at least one concentration of a target molecule;
 - f) for each contacting of said ligand with at least one concentration of said target molecule as defined in step e), collecting NMR relaxation dispersion profiles for said ligand contacted with said at least one concentration of said target molecule for every concentration of said target molecule at two or more magnetic fields;
 - g) fitting said dispersion profiles obtained in step f) by including the relaxation rates of step c) and using a two-state exchange model independently for every nucleus, and independently or simultaneously for every concentration of the target molecule; and
 - h) determining a ligand site obeying a two-state binding behavior based on feasibility of extracted R_{2b} and p_b parameters obtained by the fitting of step g), wherein R_{2b} represents a nominal transverse relaxation rate of said

ligand bound to the target molecule and p_b represents a nominal fraction of the bound ligand.

2. The method of claim 1, wherein the ligand of step a) has at least two detectable nuclei.
3. The method of claim 1 or 2, wherein the ligand in step e) is contacted with at least two concentrations of a target molecule.
4. The method of claim 3, wherein the ligand is contacted with three concentrations of target molecules.
5. A method to determine quantitatively the dissociation rate constant (k_{off}) for a transient complex of a ligand with the target molecule comprising the steps of:
 - a) Identifying a ligand site obeying a two-state binding behavior in a transient complex of a ligand with a target molecule with the method as defined in claim 1, 2, 3 or 4; and
 - b) Extracting k_{off} values for the ligand sites obeying two-site exchange mechanism, said k_{off} values being a measure of the affinity of a transient complex of the ligand with the target molecule.
6. A method according to claim 1, 2, 3, 4, or 5, wherein the ligand is a polypeptide.
7. A method according to claim 1, 2, 3, 4, 5 or 6, wherein the ligand is a ^{15}N -enriched polypeptide.
8. A method according to claim 1, 2, 3, 4, 5, 6 or 7, wherein the ligand is a mixture of polypeptides and/or molecules.

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9. A method according to claim 1, 2, 3, 4, 5, 6, 7 or 8, wherein the target molecule is a protein or a protein assembly.
10. Use of the method as defined in claim 1, 2, 3, 4, 5, 6, 7, 8 or 9 to determine amino acid residues with detectable NMR relaxation dispersion as constituting binding hot-spots.
11. Use of the method as defined in claim 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 to identify two or more ligands that can be linked together to create high-affinity molecules.
12. Use of the method of claim 1, 2, 3, 4, 5, 6, 7, 8 or 9 to study high-affinity protein-protein interactions or slow-dissociating ligand-target complexes.
13. The use of claim 10, 11 or 12, wherein the ligand is a polypeptide or a protein.
14. The use of claim 10, 11, 12 or 13, wherein the ligand is ^{15}N -enriched.
15. The method of any one of claims 1 to 9, where the NMR relaxation dispersion profiles are collected by a CPMG method.
16. The use of claims 10 to 14 where the NMR relaxation dispersion profiles are collected by a CPMG method.

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